

Increased β -aminoisobutyric acid in rat liver with 6-azauracil and its enantiomer

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When 6-azauracil was subcutaneously injected, β -aminoisobutyric acid and β -alanine contents were increased 27 and 61-fold, respectively, in rat liver. Incorporation of [methyl- ^{14}C]thymine into β -aminoisobutyric acid was increased to 42-fold by 6-azauracil treatment. The absolute configuration of this amino acid was proved to be the (*R*)-form by means of a gas-chromatographic technique. 6-Azauracil inhibited β -alanine-pyruvate aminotransferase activity with an I_{50} of approx. 2.5 mM.

β -Aminoisobutyric acid β -Alanine 6-Azauracil Thymine β -Alanine-pyruvate aminotransferase

1. INTRODUCTION

It is well known that 6-azauracil and its derivatives act as a metabolic inhibitor for RNA synthesis. 6-Azauridine 5'-phosphate is a competitive inhibitor of orotidine-5'-phosphate decarboxylase [1]. Moreover, the mode of action of 6-azauridine in its capacity as a growth inhibitor has been attributed to inhibition of UTP biosynthesis by 6-azauridine 5'-phosphate [2] or incorporation of it into RNA [3]. In [4–6], we found that β -alanine content in rat liver was remarkably increased by injection of 6-azauracil and that 6-azauracil was a competitive inhibitor of purified β -alanine-2-oxoglutarate aminotransferase (EC 2.6.1.19) from rabbit liver. Slavik et al. [7] also proposed that 6-azauridine triacetate induced hyper β -alaninemia.

β -AIB is an end product of the metabolic degradation of thymine [8,9], its absolute configuration being of the (*R*)-form [10,11]. Solem et al. [11,12] found the (*S*)-isomer present in serum, which must have a different metabolic origin than from thymine degradation. The (*R*)- and (*S*)-enantiomers of β -AIB were transaminated by dif-

ferent enzymes such as β -AIB-pyruvate aminotransferase (β -alanine-pyruvate aminotransferase, EC 2.6.1.18) and β -AIB-2-oxoglutarate aminotransferase (L-3-aminoisobutyrate aminotransferase, EC 2.6.1.22), respectively [13,14].

This report describes the increase of β -AIB caused by 6-azauracil with the concomitant decrease of the activity of β -alanine-pyruvate aminotransferase in vivo and the absolute configuration of this amino acid was proved to be the (*R*)-enantiomorph.

2. MATERIALS AND METHODS

All chemicals used were of analytical grade and purchased from Nakarai Chemicals, Kyoto, if not otherwise stated. 6-Azauracil was obtained from Sigma. [methyl- ^{14}C]Thymine was purchased from Commissariat à l'Energie Atomique, France, and β -[2- ^{14}C]alanine from New England Nuclear, USA. Pure (*R*)- and (*S*)-isomers of β -AIB were kindly provided by Professor Y. Kakimoto of School of Medicine, Ehime University, Japan.

Male Sprague-Dawley rats, weighing 120–150 g, were housed in individual screen-bottomed cages in a room maintained at $23 \pm 1^\circ\text{C}$ with 50%

Abbreviation: β -AIB, β -aminoisobutyric acid

humidity and light regulation (12 h light per day). The animals were fed on a commercial stock diet (Oriental Kobe) and water ad libitum for 1 week before the experiment to acclimatize them to the new environment.

2.1. Preparation of sample for DNA-RNA and amino acid analysis

Preparations of a DNA-RNA fraction and a sample for amino acid analysis from rat liver were according to [4,15].

2.2. Amino acid and gas-chromatographic analysis

The quantitative determination and fractionation of amino acids were performed with a preparative amino acid autoanalyzer (Hitachi KLA-3B type) and an aliquot of the β -AIB fraction was saved for estimation of radioactivity and determination of the absolute configuration of the amino acid. The radioactivity was measured with a Packard liquid scintillation counter equipped with an external standard for compensating counting efficiency. The β -AIB fraction from the preparative amino acid analyzer was desalted as shown by Ogawa et al. [16] and converted to the *N*-trifluoroacetyl-(*S*)-prolyl derivative of the methyl ester of β -AIB by the method of Bonner [17]. The enantiomers of the dipeptides were separated isothermally at 185°C using a 3 mm \times 2 m column containing 2% OV 17 on gas chromatography (Yanagimoto).

2.3. Assay of β -alanine-pyruvate aminotransferase

β -Alanine-pyruvate aminotransferase activity was assayed by following the rate of formation of malonic semialdehyde according to [18].

3. RESULTS

Table 1 shows the incorporation of radioactivity into β -AIB in rat liver 20 min after [*methyl*-¹⁴C]thymine administration. The position of the radioactivity on paper chromatography (*n*-butanol:acetic acid:water = 4:4:1) was the same as that of β -AIB. The conversion of radioactive thymine into β -AIB was increased very significantly by 6-azauracil. In livers of rats treated with 6-azauracil the radioactivity of β -AIB was 37% of

Table 1

Incorporation of [*methyl*-¹⁴C]thymine into β -AIB in rat liver

Treatment	dpm/g wet tissue	% of injection
Control	$10^3 \times (23.9 \pm 1.2)$	0.86 ± 0.05
6-Azauracil	$10^3 \times (1004.2 \pm 55.5)^a$	37.61 ± 1.59^a

Five rats were used in each experimental subgroup. 10 mg 6-azauracil was dissolved in 0.4 ml of 20% propylene glycol and subcutaneously injected into the rats 5 times at intervals of 2 h. Control animals were injected with 20% propylene glycol in the same manner. 2 h after the last injection of 6-azauracil, 10 μ Ci [*methyl*-¹⁴C]thymine (50 mCi/mmol) was injected into the femoral vein and the animals killed 20 min later. The liver was quickly removed and provided as the sample preparations. Each value is the mean \pm SE. ^a $p < 0.01$ compared to control

Table 2

Effect of 6-azauracil on β -AIB and β -alanine contents in rat liver

Treatment	μ mol/g wet tissue	
	β -AIB	β -Alanine
Control	0.040 ± 0.003	0.180 ± 0.028
6-Azauracil	0.849 ± 0.037^a	10.950 ± 0.572^a

6-Azauracil treatment and autopsy were the same as in table 1. Each value is the mean \pm SE. ^a $p < 0.01$ compared to control

the total radioactivity injected, while that in control rat liver was 0.9%. On the other hand, the radioactivities of the DNA + RNA fraction in liver of control and 6-azauracil-treated rat were 0.32 and 0.11%, respectively, of [*methyl*-¹⁴C]thymine administered.

Table 2 shows the contents of β -AIB as well as β -alanine in rat liver and the effect of 6-azauracil. When 6-azauracil was consecutively injected 5 times at intervals of 2 h, the levels of β -AIB and β -alanine were increased very significantly, 22- and 61-fold, respectively.

By application of gas-liquid chromatography, the enantiomorphs of β -AIB in liver could be determined. Authentic *N*-trifluoroacetyl-(*S*)-prolyl-(*R*)- β -AIB methyl ester was separated from the

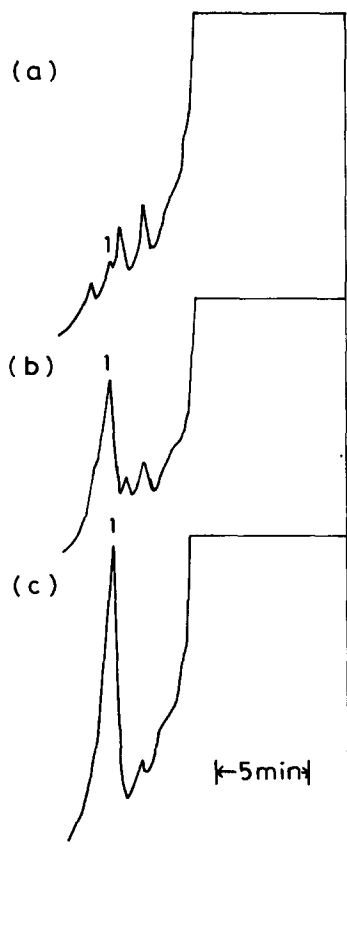


Fig.1. Gas-chromatographic determination of the β -AIB enantiomorph in liver of rat administered with 6-azauracil. 6-Azauracil was injected as described in table 1. Sample preparation and gas-chromatographic conditions are described in section 2. (a) Control rat liver, (b) 6-azauracil-treated rat liver, (c) (b) + authentic *N*-trifluoroacetyl-(*S*)-prolyl-(*R*)- β -AIB methyl ester.

derivative of the (*S*)-isomer (not shown). As shown in fig.1, the striking accumulation of the (*R*)-enantiomer of β -AIB was observed in liver of rat injected with 6-azauracil and the peak of the derivative of the (*R*)-isomer was increased by the addition of the authentic one. Thus, it is apparent that the increase of liver total β -AIB was caused by the (*R*)-enantiomorph only.

Fig.2 shows the inactivation of β -alanine-pyruvate aminotransferase activity by 6-azauracil. The enzyme activity in raw extract of rat liver was

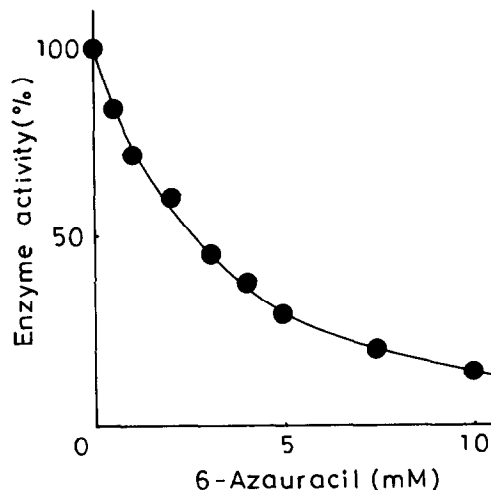


Fig.2. Effect of 6-azauracil on β -alanine-pyruvate aminotransferase activity in rat liver. Rat liver was homogenized in 10 mM potassium phosphate, pH 7.5, containing 1 mM EDTA, 2 mM 2-mercaptoethanol and 40 μ M pyridoxal 5'-phosphate. The homogenate was centrifuged for 20 min at $27000 \times g$ and the supernatant used for the estimation of enzyme activity. Relative activity is defined as the percentage of the activity compared to that in the absence of 6-azauracil.

0.17 ± 0.02 nmol/min per mg protein under the standard assay conditions. In the presence of 1 mM β -alanine and 10 mM pyruvate, 6-azauracil inactivated β -alanine-pyruvate aminotransferase activity with an apparent I_{50} of 2.5 mM.

4. DISCUSSION

Here, the (*R*)-isomer of β -AIB was accumulated in rat liver by 6-azauracil treatment (table 2, fig.1). These results show that 6-azauracil increased one of the metabolites of thymine. The incorporation of radioactivity from [*methyl*- 14 C]thymine into β -AIB in liver was increased 42-fold by 6-azauracil. Furthermore, 6-azauracil inhibited uridine 5'-phosphate synthesis [1] and decreased the incorporation of [*methyl*- 14 C]thymine into DNA. From these results, it may be proposed that 6-azauracil does not enhance pyrimidine biosynthesis, but that it inhibits the metabolism of (*R*)- β -AIB. As shown in fig.2, 6-azauracil inactivated β -alanine-pyruvate aminotransferase activity in rat liver.

In [6,18], we found that β -alanine was mainly transaminated by β -alanine-2-oxoglutarate ami-

notransferase in liver and that 6-azauracil inhibited the purified enzyme activity. In this paper we also found that 6-azauracil inactivated β -alanine-pyruvate aminotransferase activity to accumulate (*R*)- β -AIB. Thus, 6-azauracil may facilitate the study of pyrimidine metabolism including β -alanine and (*R*)- β -AIB.

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